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Review on Analytical Techniques used for the Estimation and Persistence of Ready-Mix Formulation Residues in Various Crops and Soil

Anita[1], Bharti Sharma[2]

[1]Department of Chemistry and Biochemistry, CCS Haryana Agricultural University, Hisar-125004, Haryana, India

[2]Department of Bio-Nanotechnology, G.J. University of Science & Technology, Hisar-125001, Haryana, India

Abstract: ***In the existing review, an effort has been made to evaluate different type of techniques used for residues quantification in vegetable crops and soil. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) methodologies have been applied in many laboratories, with good results and high recoveries, for several complex matrices extractions, such as: vegetables, fruits, biological sample, food products and soil samples. Vegetables were analyzed for pesticide residues using multiresidue analysis by High-Performance Liquid Chromatography (HPLC) equipped with UV detector. To analyze the selected pesticides and the degradation products, different analytical methods were used gas chromatography coupled with a mass spectrometer (GC-MS/MS), ultra performance liquid chromatography coupled with a mass spectrometer (UHPLC-MS/MS). So in this study QuEChERS, HPLC, GLC, GC-MS/MS, UHPLC-MS/MS etc. techniques are reviewed.***

Keywords: Pesticides, Residues, QuEChERS, HPLC, GLC, GC-MS/MS, UHPLC-MS/MS

1. Introduction

The indiscriminate use of broad spectrum synthetic pesticides has resulted in reduction of biodiversity, outbreak of secondary pests, development of pesticide resistance, pesticide-induced resurgence and contamination of food and the ecosystem. However, the use of synthetic pesticide as crop protection chemical cannot be fully substituted in modern agriculture for assured crop production. Enhancement of crop production can be achieved only by the application of new pesticides. Ready-mix formulations are cost effective and have high efficiency of controlling the pest as compared to single insecticide. The pre-combination pesticide mixtures appear to be advantageous than single compound or tank mixtures since these give broad spectrum of activity, additive joint action, economic in pest control and application, multiple mode of action and overcoming delayed resistance to pesticides [1]. But it is of great concern to check the amount of residues persisted in food products as well as in soil and water bodies. For this purpose different types of techniques viz. GLC, HPLC, GC-MS etc. used in laboratories are reviewed below.

2. Materials and Method

Pesticide residues analysis in different crop samples is typically based on methods that include multiple steps: extraction, clean-up and finally determination of residues by gas or liquid chromatography. In the present studies, extraction of pesticides is the first step in the sequence of events to determine pesticide residues from various sample matrices. Pesticides along with other pigments and unwanted material are also extracted into solvent. In order to avoid interferences by co-extractives such as plant pigment, waxes and fats present in the extract, isolation of the toxicant is achieved before taking up estimation of pesticide residues. To attain the essential sensitivity, the interfering substances should be removed from the pesticides with one or more procedures, which is commonly known as clean-up. The degree of clean-up requirement depends on the scope of analysis, the complexity of the samples and the sensitivity of detection methods available for the contaminant sought [2]. Different solvents used for selective extraction of ready-mix formulation from plant and soil matrix following different extraction procedures, clean-up and quantification methods viz. GC, HPLC etc. reported by various workers have been reviewed.

3. Estimation of residues in vegetables

Residues of novaluron in tomato at dosage of 37.5 g a.i. ha^{-1} was investigated [3] the representative tomato samples were collected at 0 (2 h after spraying), 1, 3, 7, 9, 12, and 14 days after treatment. Agilent 1260 series HPLC with diode array detector (DAD) and Zorbax XDB C18 (4.6 m x 250 mm x 5 µm) column was used for separation and quantitative analysis of novaluron in tomato samples. The mobile phase was methanol and water (80:20, v/v), flow rate of the mobile phase was 1 ml/min, oven temperature during the analysis was 25˚C and quantification was done at 254 nm. The method was validated using blank samples spiked at three levels and results showed that recoveries ranged from 93 to 99%. Novaluron residues tend to dissipated following first-order rate kinetics with half-life of 2.08 days.

Indoxacarb residues in cabbage were determined by [4] at dosage of 27 g/ha for foliar application. Samples were collected after treatment at 0 (2h), 1, 2, 3, 5, 7, 10, 14, 21, 30 and 45 days after treatment. The chromatographic determination was performed by using Waters HPLC system equipped with a 2695 separations module and a 2998 photodiode array detector (PAD). The enantiomers of indoxacarb were separated on a Phenomenex Lux cellulose-1 column filled with CSP of cellulose-tris-(3,5-dimethylphenylcarbamate) (CDMPC) (250 m x 4.6 mm x 5 mm). The mobile phase was a mixture of

hexane/isopropanol (85/15, v/v) and flow rate was 0.8 ml/min. Photodiode array detector (PAD) detection was conducted at 310 nm at room temperature. Dissipation of indoxacarb enantiomers followed first-order kinetics in cabbage. The half-lives of two enantiomers in cabbage ranged from 2.8 to 4.6 days.

[5] Studies of persistence of cypermethrin, deltamethrin, profenofos and triazophos in cauliflower curd after application of two premix formulation viz, Roket 44EC (profenofos 40% + cypermethrin 4%) and Anaconda Plus 36 EC (triazophos 35% + deltamethrin 1%) at recommended single (1.0 L/ha) and double (2.0 L/ha) doses. In the case of Roket 44 EC, residues of cypermethrin dissipated with the half-life values of 1.5-2.1 days, whereas residues of profenofos dissipated with the half-life of 2.9-3.3 days in cauliflower curd. In the case of Anaconda Plus 36 EC residues of triazophos and deltamethrin dissipated from curd with the half- life values of 2.6-3.0 and 2.2-2.6 days, respectively.

Residues of organophosphates in cauliflower were cleaned by adopting column chromatographic technique. Column was packed with silica gel and activated charcoal (5:1 w/w) in between the layers of anhydrous sodium sulphate. Extract was eluted with 125 ml mixture of acetone : hexane (3:7 v/v). Final volume was made to 2ml for analysis by gas liquid chromatography (GLC). Residues of monocrotophos (2.01-3.24 µg g^{-1}), parathion (5.99-7.99 µg g^{-1}), pendimethalin (0.29-0.41 µg g^{-1}), endosulphan-II (0.70-1.64 µg g^{-1}), captafol (0.31-0.51 µg g^{-1}), permethrin (0.25-0.35) and cypermethrin (0.40-0.60 µg g^{-1}) were detected in cauliflower by [6].

Takkar [7] determined the residues of indoxacarb on cauliflower curds and estimated by gas chromatograph-mass spectrometer (GC-MS) in selective ion monitoring (SIM) mode. The gas chromatograph (Shimadzu-QP 2010) with auto injector, equipped with mass spectrometer and capillary column Rtx-5 Sil MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness) was used to verify the results. The average initial deposits of 0.23 and 0.45 mg kg^{-1} were observed after last application of indoxacarb @ 52.2 and 104.4 g a.i. ha^{-1} at single and double dose, respectively. The residues in cauliflower dissipated below its LOQ 0.01 mg kg^{-1} after 7 days and its half-life periods were observed to be 1.12 and 1.31 days, at single and double the dosages, respectively.

Gupta [5] sprayed tomato crop with pre-mix formulation *viz*. Roket 44 EC (profenofos 40% + cypermethrin 5%) and Action-505 EC (chlorpyriphos 50% + cypermethrin 5%) at fruiting stage. Pre washing was done with n-hexane and extract was eluted with mixture of n-hexane : acetone (8:2 *v/v*), concentrated and analyzed on Varian CP 3800 GLC equipped with ^{63}Ni (ECD) and CP-Sil 5CB (25 m × 0.52 mm × 0.25 µm). Half-life value of cypermethrin in Roket 44 EC varied from 2.0 - 3.6 days whereas residues of profenophos in Roket 44EC dissipated with half-life of 2.2 - 5.4 days. In case of Action 505 EC, residues of chlorpyriphos and cypermethrin dissipated from fruits with half-life values of 2.9 - 3.3 and 2.5 - 4.8 days, respectively.

Persistence and efficacy at different doses of bifenthrin (25 and 50 g a.i. ha^{-1}), fipronil (50 and 100 g a.i. ha^{-1}) and indoxacarb (70 and 140 g a.i. ha^{-1}) has been studied in okra fruits by [9]. Residues of bifenthrin, fipronil and indoxacarb were analysed on Varian CP 3800 gas liquid chromatography (GLC) equipped with electron capture detector and CP-Sil 5 CB (25 m x 0.25 mm x 0.25 µm) column. Flow rate of nitrogen was 2 mL per min. The retention time was 6.02 min for bifenthrin, 3.81 min for fipronil and 5.64 min for indoxacarb. Limit of detection of fipronil, bifenthrin and indoxacarb was 0.010, 0.015 and 0.020 ng, respectively. Recovery of bifenthrin, fipronil and indoxacarb from okra fruits were fortified at 0.5 µg g^{-1} and varied from 83 to 91, 86 to 89 and 88 to 92% respectively. Results showed that residues persisted up to 10 days with half-life of 1.32-1.58 days for bifenthrin, 0.65-1.12 days for fipronil and 0.58-1.02 days for indoxacarb. Based on acceptable daily intake (ADI) suggested waiting period was 1 day for both bifenthrin and indoxacarb and 3 days for fipronil.

Vegetables like egg plant, pumpkin and okra were analyzed for pesticide residues of organophosphate group like triazophos, profenophos and chlorpyriphos using multiple residue analysis by high-performance liquid chromatography (HPLC) equipped with UV detector and HPLC analyses were performed on gasocratic system using a Shimadzu chromatograph including LC-10 AS pumps, 20-11 reodyne injector, SPD-10A UV detector operating at 208 nm and supelco C-18 analytical column [25 cm x 4.6 mm (i.d)]. A mixture of water and acetonitrile (ACN) was used as mobile phase. The flow rate was kept at 1 mp/min by [10].

Sharma and Mohapatra [11] determined the persistence of indoxacarb residues in tomato and thiamethoxam residues in okra foliar, after application of indoxacarb @ 0.5 and 1.0 ml/l and thiamethoxam @ 0.2 and 0.4 g/l. The residues of indoxacarb and thiamethoxam were estimated by HPLC (Shimadzu, LC-6A Model) equipped with a UV-VIS detector. For indoxacarb residues, retention time was 4.2 min. and for thiamethoxam retention time was 3.9 min. The limits of detection were found to be 0.001 ppm in both cases. The recovery tests carried out at fortification level of 0.1 ppm of indoxacarb and thiamethoxam in tomato and okra, respectively and showed that the recoveries ranged from 92-95% of indoxacarb residues in tomato and 84-85% of thiamethoxam residues in okra. The residues of the insecticides dissipated fast to below detectable limits within 7-10 days after their last application. The residues dissipated with half lives of 1.1 to 1.5 days and the pre harvest intervals calculated on the basis of respective MRL value was 1 day only for both the insecticides.

Figure 1: High Performance Liquid Chromatography

Figure 2: Gas Liquid Chromatography

4. Estimation of residues in soil

According to Sun [4] determined indoxacarb residues in soil at dosage of 27 g/ha under cauliflower crop. Samples were collected after treatment at 0 (2h), 1, 2, 3, 5, 7, 10, 14, 21, 30 and 45 days after treatment. The soil was sampled from 0–10 cm depth. Chromatographic determination was performed using Waters HPLC system equipped with a 2695 separations module and a 2998 photodiode array detector (PAD). The initial concentration of (-)-indoxacarb in Beijing soil was 0.51 mg/kg and decreased to 0.37 mg/kg in 30 days. The degradation rate was 73%, whereas the initial concentration of its antipode was 1.71 mg/kg which decreased to 0.68 mg/kg in 30 days. Degradation rate of (+)-indoxacarb in the Anhui soil followed first-order kinetics. The concentration of (+)-indoxacarb degraded from 4.68 to 0.70 mg/kg within 45 days. Whereas, the initial concentration of (-)-indoxacarb was 1.63 mg/kg, increased to 1.7mg/kg in 2 days and then decreased with time. The half-lives of (-)-indoxacarb and (+)-indoxacarb was 32 and 21 days, respectively, in the Anhui soil. These results showed great difference in the degradation rates between two enantiomers.

Takkar [7] determined the residues of indoxacarb in soil at three treatments, control (T_1), single dose @ 52.2 (T_2) and double dose @ 104.4 g a.i. ha^{-1} (T_3). The soil samples of about 1 kg were collected from each plot separately after 15 days following the last application. The cleaned up extracts were analysed by gas liquid chromatography (Perkin Elmer Clarus 500) equipped with an electron capture detector (ECD) ^{63}Ni operated at 310°C. Chromatographic separation was carried out using a capillary column Elite 608 (50 m × 0.53 mm i.d, 1.5 µm film thickness) held at 290°C with spilt ratio 1:10 for the estimation of indoxacarb residues. Flow rate of nitrogen was 2 ml min^{-1}. The injector port and detector was held at 300°C and 310°C respectively. Retention time observed for indoxacarb was 6.20 minutes. Recoveries were found to be consistent and more than 80% at different concentration (0.01, 0.05, 0.1, 0.2 mg kg^{-1}) with relative standard deviation (RSD) below 15% confirmed a good repeatability of the method.

The residues of multipesticide were extracted from the soil by Samriti [12]. In soil samples 0.5 ml of ammonia solution was added and kept for half hour. Then added 0.3 g of florisil, activated charcoal and 10 g anhydrous sodium sulphate mixed them thoroughly and packed in a glass column and elute the column with 125 ml of hexane : acetone (9:1 *v*/*v*) solution. Concentrated the elute and analyzed on gas liquid chromatography (GLC) Model Shimadzu - 2010, equipped with ECD (Ni^{63}), split injection system and fused capillary column (SPB-5) 30 m × 0.32 mm i.d., 0.25 µm film thickness of polysiloxane (5% diphenyl, 95% dimethyl). The half-life period was reported to be in the range of 1.90 to 4.53 days at single and double dose, respectively, in soil under okra crop.

Persistence of cypermethrin, chlorpyriphos and profenofos in soil underneath tomato following application of two pre-mix formulations of insecticides *viz*. Roket 44EC (profenofos 40% + cypermethrin 5%) and Action-505 EC (chlorpyriphos 50% + cypermethrin 5%) was studied by [9] and for extraction, soil samples were mixed with 200 mg of 1:1 mixture of charcoal and florisil and packed in the glass column in between two layers of sodium sulphate. The column was eluted with 125 ml of hexane : acetone (8:2 *v*/*v*). The extract was concentrated under reduced pressure. No further clean-up was required for soil samples. Residues were analyzed on Varian CP-3800 gas liquid chromatography (GLC) equipped with electron capture detector (ECD) and CP-Sil 5CB (25 m × 0.25 mm × 0.25 µm) column. In soil, residues of profenofos persisted for 7-15 days, whereas residues of chlorpyriphos and cypermethrin persisted for 0-7 days.

Das [13] applied novaluron (99.5 % pure) to soil at field rate (FR), 2 times FR (2 FR) and 10 times FR (10 FR). Pesticide degradation was studied in aerobic state for both non-sterile and sterile conditions under dark in triplicate for each soil. 50 g sample was extracted with 100 ml of acetonitrile : water (65 : 35) using a mechanical shaker. High Performance Liquid Chromatography (HPLC 1050 Hewlett Packard equipped with UV detector and Chemito 5000 Data Processor) was used for final determination of novaluron residues. Shandon Hypersil (250 x 4.6 mm ODS x 5 µm (RPC_{18})) column was used for the chromatographic separation of novaluron. The average recovery was 89.6 - 91.3% for novaluron from both types of soils. The half-lives of novaluron in non-sterilized soils ranged from 17.0 - 17.8

days (alluvial soil) and 11.4 - 12.7 days (coastal saline soil), while the values in case of the sterilized soils were 53.7 - 59.0 days (alluvial soil) and 28.9 - 29.8 days (coastal saline soil), respectively.

A rapid and accurate method for the extraction and determination of the two organophosphorous insecticides viz, chlorpyriphos and acephate in top and subsoil materials of three tropical clayey soil was developed by [14]. Pesticides were determined by GC-FPD. The method did not require clean-up of the extracts prior to GC analysis and could be detected down to 0.01 mg kg^{-1}.

5. Conclusion

It was concluded that to analyze the selected pesticides and the degradation products, different analytical methods were used in which gas chromatography coupled with a mass spectrometer (GC-MS/MS) and ECD for pesticides and HPLC for herbicide was most benefitial in residue analysis at micro quantities.

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